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VACCINATION OF MONKEYS AGAINST PNEUMOCOCCUS TYPE I PNEUMONIA BY MEANS OF INTRATRACHEAL INJECTION OF PNEUMOCOCCUS TYPE I VACCINE.[1]

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In a previous study (1) it has been shown that monkeys can be completely immunized against pneumococcus Type I pneumonia by three subcutaneous injections of pneumococcus Type I vaccine. The results with pneumococcus vaccine in monkeys were in harmony with those obtained in man by Cecil and Austen (2), and by Cecil and Vaughan (3), who tested the prophylactic value of pneumococcus vaccine on recruits in the United States Army and found that the incidence rate of pneumonia was markedly lowered in organizations that had received subcutaneous inoculations of pneumococcus vaccine. These investigators, however, commented on the severe reactions sometimes produced by pneumococcus vaccine, and intimated that improvements in methods of preparation and administration would have to be forthcoming before active immunization against pneumonia would be practical in civil life.

During the past three years a number of modified pneumococcus vaccines have been tried by the authors of this report, but none of them has been quite so efficient as the original saline suspension of killed pneumococci. With regard to modifications in the method of administration, it occurred to us that a satisfactory immunity against pneumonia might be obtained by injecting the vaccine directly into the trachea. Such a procedure seemed entirely rational when taking into consideration the fact that in lobar pneumonia infection takes place through the trachea, and in the very early stages is a peritracheal and peribronchial infection. Furthermore, the work of Besredka (4) on the production of local immunity in the respiratory tract by intratracheal injections of vaccine, and the more recent report of Mackenzie and Baldwin (5) on the desensitization of hay-fever patients by the local application of pollen extracts to the nasal mucous membrane, lent support to such a proposition.

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[1] From the Hygienic Laboratory of the United States Public Health Service and the second (Cornell) medical division and pathological laboratory of Bellevue Hospital, New York. This work also received financial aid from the Metropolitan Life Insurance Co.'s influenza fund.

The experiments reported in this article all have to do with intratracheal vaccination. In these experiments two species of monkeys have been used, *Macacus syrichtus* and *Cebus capucinus.* The vaccine was prepared in the usual way and was similar in every respect to the pneumococcus saline vaccine employed in preceding experiments. The vaccine was prepared from a highly virulent strain of pneumococcus Type I, which was capable of killing a mouse in doses of 0.00000001 c. c. of a 24-hour broth culture. The technique used for vaccinating monkeys intratracheally was similar to that which had been previously developed for producing experimental pneumonia in monkeys by the intratracheal injection of virulent pneumococci. The neck of the animal was shaved and the skin painted with iodine. The vaccine was then injected directly into the trachea with a sterile hypodermic syringe. Immediately after the injection of vaccine the operating board was elevated in order to facilitate descent of the vaccine into the lungs. The injections were given at intervals of five to seven days, and the immunity of the monkeys was tested two or three weeks after the third administration of vaccine by inoculating the immunized monkeys intratracheally with small doses (0.001 to 0.0001 c. c.) of living virulent pneumococcus culture.

EFFECT OF THREE LARGE DOSES OF PNEUMOCOCCUS VACCINE INJECTED INTRATRACHEALLY.

The object of the first experiment was to determine whether three large doses of pneumococcus vaccine injected intratracheally would afford adequate immunity against a subsequent pneumonia. The total dosage of vaccine used was exactly equivalent to that which had been successfully employed in the subcutaneous vaccination experiments, namely, 120,000,000,000 pneumococci.

Experiment 1.—Three Philippine monkeys were vaccinated intratracheally, as follows:

May 18, 1921, No. 122 (2140 gm.), No. 123 (2546 gm.), and No. 124 (2090 gm.) received each 20,000,000,000 (2 c. c.) of pneumococcus Type I vaccine intratracheally. May 25 each monkey received 40,000,000,000 (2 c. c.) of pneumococcus Type I vaccine intratracheally. June 1 each monkey received 60,000,000,000 (2 c. c.) of pneumococcus Type I vaccine intratracheally. There were no noticeable reactions to any of the three inoculations. June 22, three weeks after the last injection of vaccine, each of the three monkeys received 0.001 c. c. of a virulent 18-hour broth culture of pneumococcus Type I intratracheally. At the same time an unvaccinated control monkey, No. 140 (2600 gm.), received the same amount (0.001 c. c.) of culture intratracheally.

The results of this experiment are shown in Figure 1. The three vaccinated monkeys remained perfectly well. They showed no rise in temperature, and only a slight and temporary rise in the leucocyte

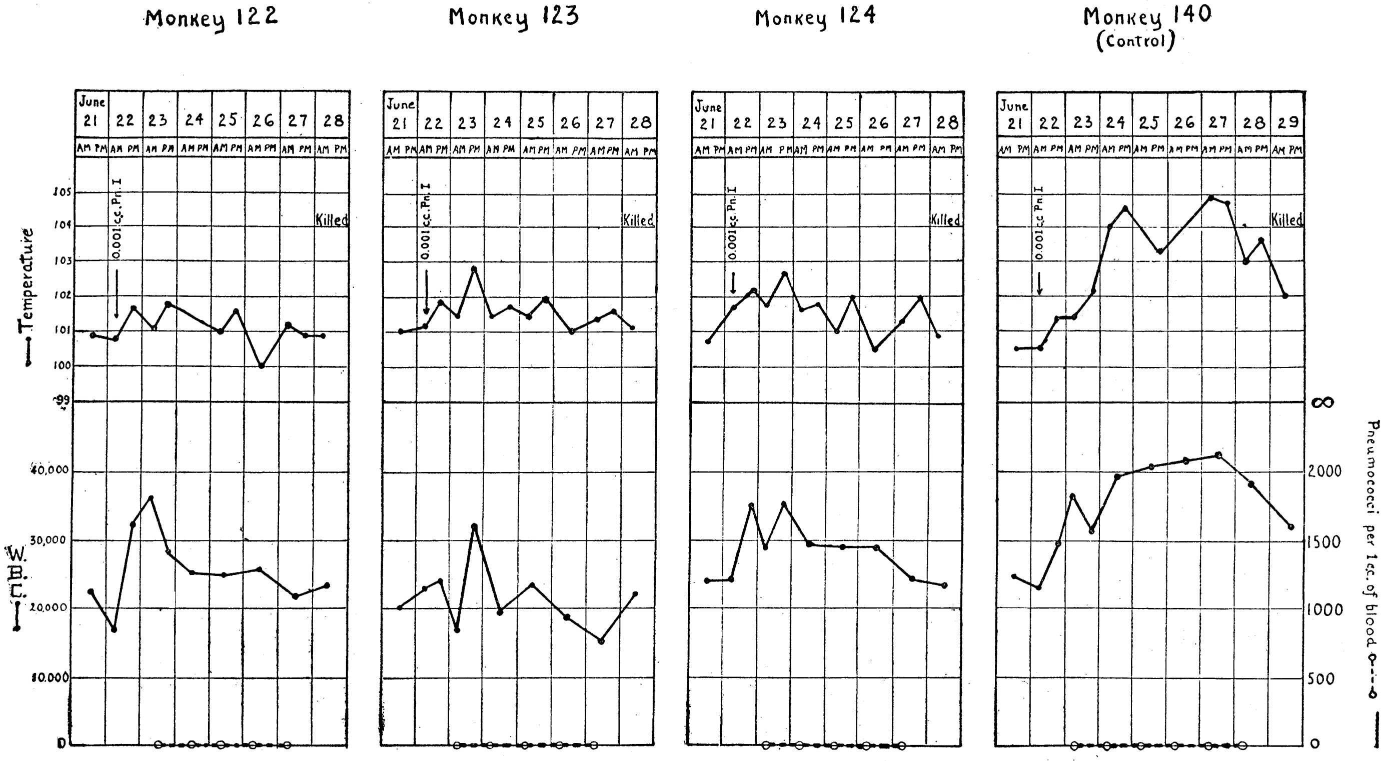

FIG. 1.—Active immunity against pneumococcus Type I pneumonia, following three large intratracheal injections of pneumococcus Type I vaccine. Monkeys 122, 123, and 124 each received 120 billion pneumococcus Type I vaccine intratracheally. Monkey 140 was the control.

count. The blood remained sterile in all three vaccinated monkeys. The control monkey became ill shortly after inoculation with the virulent pneumococcus and ran a typical course of pneumonia with temperature of 103° to 105° F., leucocytes 30,000 to 43,000, and lysis on the eighth day. The three vaccinated monkeys were killed on the seventh day, the control on the eighth day, after inoculation. The lungs were entirely normal and sterile in all three vaccinated monkeys. There was no evidence in the gross or in microscopic sections of a past or present pneumonia. The control monkey showed a perfectly typical lobar consolidation of the right lower lobe in the early stage of resolution. Pneumococcus Type I was recovered in cultures from the consolidated lobe.

Protection tests.—The three vaccinated monkeys were bled just before their active immunity was tested, in order to determine the protective power of their serums in mice. No protective substance against pneumococcus Type I could be demonstrated in any of the serums, even when doses of culture as minute as 0.0000001 c. c. were used.

From this experiment it is evident that three large intratracheal doses of pneumococcus Type I vaccine conferred complete protection against a subsequent pneumococcus Type I pneumonia. The actual dose of vaccine used was the same as that employed in the subcutaneous experiments previously reported.

EFFECT OF THREE SMALL DOSES OF PNEUMOCOCCUS VACCINE INJECTED INTRATRACHEALLY.

It was desirable to determine whether an adequate immunity could be established by the intratracheal injection of smaller doses of vaccine. A second experiment was therefore carried out in which the total dosage of vaccine injected was equivalent to approximately one-tenth of the total dosage employed in the first experiment.

Experiment 2.—Three Philippine monkeys were vaccinated as follows:

January 30, 1922, No. 177 (2180 gm.), No. 178 (2088 gm.), and No. 181 (1572 gm.), received each 600,000,000 (2 c. c.) of pneumococcus Type I vaccine intratracheally. February 4, each monkey received 5,000,000,000 (2 c. c.) of pneumococcus Type I vaccine intratracheally. No. 177 escaped from cage just after the first vaccination and burnt his feet on a hot radiator. At this time (February 4) there were large blebs on all four feet, some of which had ruptured and were oozing blood. February 9, each monkey received 7,000,000,000 (2 c. c.) of pneumococcus Type I Vaccine intratracheally. Feet of monkey 177 were still sore and oozing bloody fluid. Feet washed with bichloride of mercury (1–1000) solution. In spite of this treatment, however, the feet became infected, and when the tests for immunity were carried out two weeks later, they were still swollen and sore, and the monkey was running a temperature of 103° to 104° F. February 25, 16 days after the last injection of vaccine, each of the three vaccinated monkeys received 0.0001 c. c. of a virulent 18-hour culture of pneumococcus Type I intratracheally. At the same time, an unvaccinated control monkey, No. 183 (2434 gm.), received the same amount (0.0001 c. c.) of culture intratracheally.

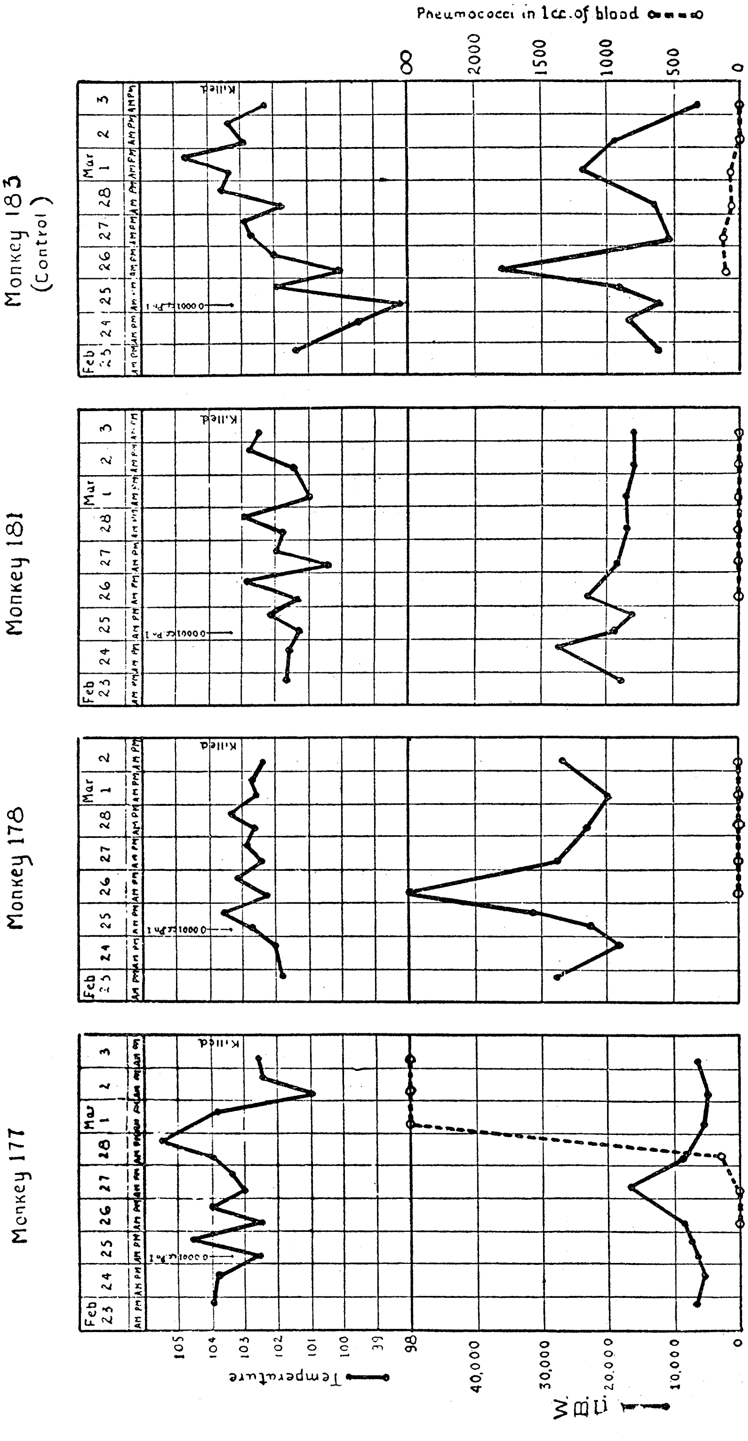

FIG. 2.—Active immunity against pneumococcus Type I pneumonia, following three small intratracheal injections of pneumococcus Type I vaccine. Monkeys 177, 178, and 181 each received 12.6 billion pneumococcus Type I vaccine intratracheally. Monkey 183 was the control.

The results are indicated in Figure 2. Monkey No. 177, the vaccinated monkey, whose feet had been accidentally burned and thereafter had become infected, gradually developed signs of pneumonia. His temperature rose slowly to 105.4°, and blood cultures showed a severe pneumococcus septicemia. On the seventh day following inoculation he was killed by a blow on the head. Autopsy revealed lobar pneumonia of the right upper and right lower lobes, in the early stage of gray hepatization. Pneumococcus Type I was recovered in pure culture from the consolidated areas and also from the heart's blood. The other two vaccinated monkeys, Nos. 178 and 181, remained healthy in spite of the inoculation. There was little or no change in their temperature, nor could pneumococci be demonstrated at any time in their blood. These two monkeys were killed, one on the sixth day and the other on the seventh day following inoculation, and at the autopsies normal sterile lungs were found in both cases.

The control for this experiment, monkey No. 183, developed the symptoms and signs of pneumonia and, when killed on the seventh day after inoculation, showed a small patch of consolidation in each upper lobe. Pneumococcus Type I was recovered from the consolidated areas, and Gram positive diplococci were demonstrated in sections of the lung stained by the Gram method.

Protection tests.—Blood was withdrawn, as usual, from the vaccinated monkeys, just before they were inoculated with living pneumococci, to test for protective bodies against pneumococcus Type I. No protection could be demonstrated in the serums of No. 177 or No. 178. In the case of No. 181, the mice receiving the two smallest injections of culture (0.00000001 c. c. and 0.0000001 c. c.) both survived.

This experiment indicates that an adequate immunity against pneumococcus Type I pneumonia can be established in monkeys with doses of vaccine only one-tenth as large as those previously used, provided the vaccine is given intratracheally. To be sure, monkey 177 failed to develop sufficient immunity to protect him against a subsequent pneumonia, but this failure was probably referable to the infection of the feet which existed at the time the monkey's immunity was tested.

EFFECT OF PNEUMOCOCCUS VACCINE SPRAY.

If monkeys could be protected against pneumococcus pneumonia by three small intratracheal injections of pneumococcus vaccine, there was some ground for hoping that a satisfactory immunity might be produced by the mere inhalation of a vaporized pneumococcus vaccine. Accordingly, three monkeys were sprayed every day for approximately three weeks with pneumococcus vaccine, in order to determine whether this method of vaccination was practical.

EXPERIMENT 3.—Three Philippine monkeys were sprayed with pneumococcus vaccine, as follows:

October 4, 1921, No. 149 (1982 gm.), and No. 150 (1814 gm.) received each 1 c. c. pneumococcus saline vaccine (20,000,000,000 pneumococci per c. c.) in the form of vapor from a De Vilbiss atomizer directly into the pharynx. Artificial respiration was practiced on the monkeys in such a way that deep inspiration occurred synchronously with each squeeze of the atomizer bulb. The monkeys, however, offered considerable opposition to this procedure and, by blocking off the nasopharynx with the tongue and soft palate, made it quite difficult to get the vaccine into the trachea. This treatment was repeated every day for 24 days with dosage of vaccine gradually increasing. November 10, 1921, monkey No. 152 (1467 gm.) was sprayed with pneumococcus Type I vaccine in a similar manner and the treatment repeated every day for 19 days. November 9, 1921, twelve days after the completion of vaccination, each of the three monkeys received 0.001 c. c. of a virulent 18-hour broth culture of pneumococcus Type I intratracheally. At the same time an unvaccinated control monkey, No. 155 (2032 gm.), received the same amount (0.001 c. c.) of culture intratracheally.

The results of this experiment are shown in Figure 3. The three vaccinated monkeys and the control all developed pneumonia. The two monkeys, however, that had been sprayed every day for 24 days (Nos. 149 and 150) remained lively during the entire period of observation and never developed a positive blood culture. No. 152 had been sprayed only nineteen times. He developed a lobar pneumonia with leucocytosis and positive culture, but was recovering when killed on the seventh day. The control ran a typical course of lobar pneumonia, as seen in monkeys, with prostration, high temperature, leucocytosis, and severe pneumococcus septicemia, and recovered by lysis on the seventh day.

Monkey 149 and monkey 150 were killed on the sixth day; monkey 152 and monkey 155 on the seventh day following the intratracheal injection of culture. Monkey 149 showed a lobar pneumonia of the right lower lobe, from which pneumococcus Type I was recovered. Monkey 150 also presented a lobar pneumonia in the gray stage, which involved the right lower lobe. Pneumococcus Type I was isolated in pure culture from the lobe involved. Monkey 152 showed at autopsy an interstitial pneumonia of both lower lobes. Cultures from the lungs yielded pneumococcus Type I. The control monkey (No. 155) was also found to have interstitial pneumonia of the right and left lower lobes, cultures from which showed a pure growth of pneumococcus Type I.

Protection tests were carried out on mice with the serums of the monkeys that had been sprayed with pneumococcus vaccine. No protective substance could be demonstrated in any of the three.

DISCUSSION.

From these experiments the inference seems justified that the intratracheal injection of pneumococcus vaccine affords just as satisfactory an immunity against pneumonia as that induced by sub-

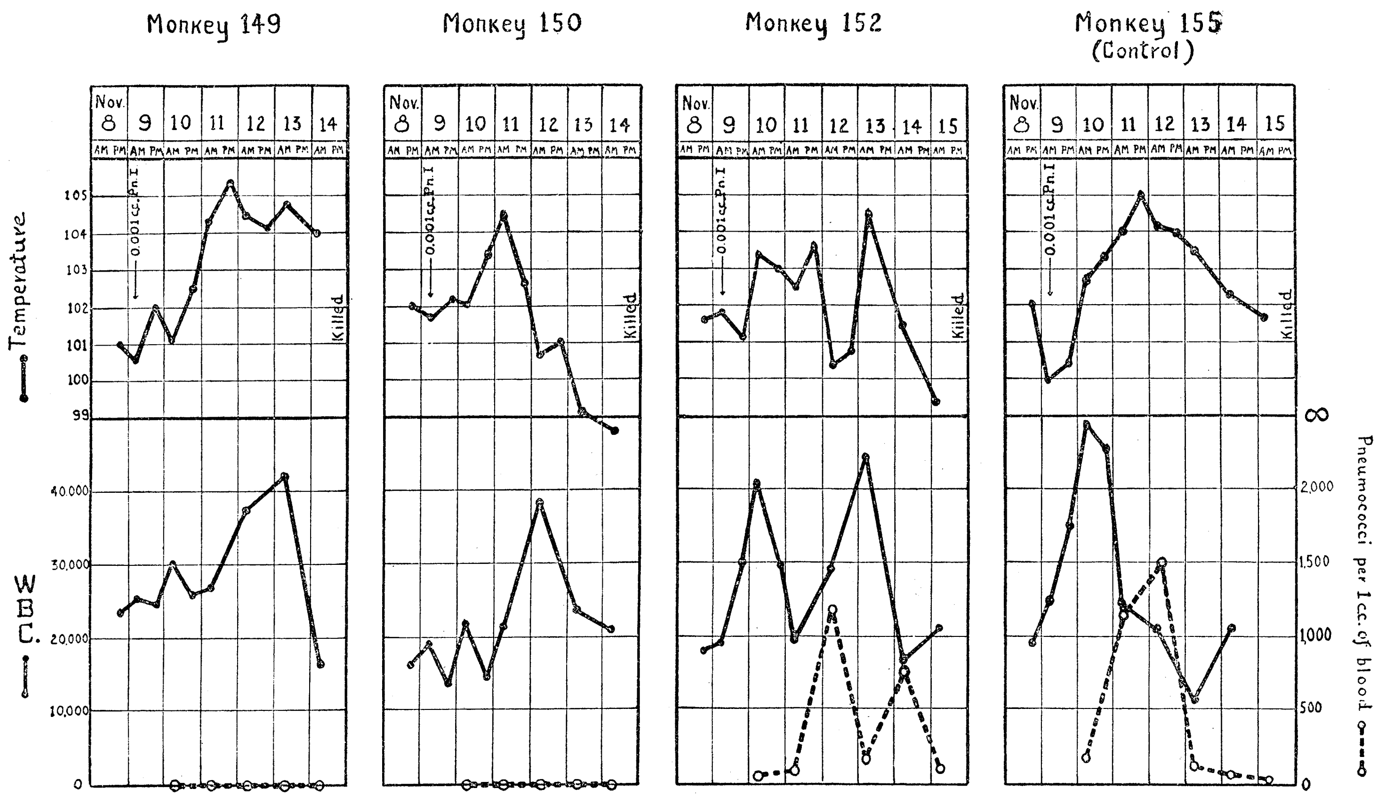

FIG. 3.—Effect of pneumococcus Type I vaccine spray. Monkeys 149, 150, and 152 were sprayed daily with pneumococcus Type I vaccine for from 19 to 24 days. Monkey 155 was the control.

cutaneous or intravenous injections. Indeed, the successful immunization of monkeys with three small intratracheal doses of vaccine indicates that immunity is more readily induced by the intratracheal route than by the subcutaneous route.

It is interesting that such a high degree of immunity could be produced by a formed antigen. The killed pneumococci are probably taken up rapidly by the epithelial cells exactly as foreign bodies are taken up. Theoretically, an antigen in solution would seem to be better adapted for establishing a local immunity such as this appears to be.

The failure of one of the vaccinated monkeys in the second experiment to resist infection serves to bring out an interesting point as to the effect of one infection on immunity against another infection. At the time when this monkey's immunity against pneumonia was being tested, he was suffering from infected feet. This apparently trivial infection was enough to neutralize completely his immunity against pneumonia. Cecil and Vaughan made a similar observation on the troops at Camp Wheeler. They found that pneumococcus vaccine would not always prevent pneumonia in patients suffering with severe attacks of influenza. The development of a high grade of active immunity against pneumonia is probably retarded in like manner by chronic systemic diseases, such as nephritis, cardiac disease, etc.

The failure of the pneumococcus vaccine spray to produce complete immunity against pneumonia was a disappointment; but it was a result not to be wondered at when subsequent experiments with india ink showed that monkeys would allow little or none of the vaporized fluid to enter the trachea. This difficulty, of course, would not be encountered in vaccinating man by the spray method. In spite of the large doses of vaccine which were administered in the first experiment, the monkeys manifested no signs of discomfort following the inoculations. Similar doses when administered subcutaneously in the previous experiments had, on some occasions, excited rather severe local reactions.

The immunity induced by the intratracheal injection of pneumococcus vaccine is probably in great part a cellular immunity. Protection tests were carried out with the serums of all the monkeys vaccinated intratracheally, and in only one case could any protective substance against pneumococcus be demonstrated. It would be interesting to know how much virulent pneumococcus culture could be tolerated intravenously in these monkeys that had been vaccinated intratracheally. It has been shown in a previous study (1) that the intravenous injection of pneumococcus vaccine confers complete immunity against intratracheal infection. The question whether or not the reverse holds true can not be answered at the present time.

CONCLUSIONS.

1. The intratracheal inoculation of monkeys with three doses of pneumococcus Type I vaccine renders them completely immune against experimental pneumococcus Type I pneumonia.
2. The mere spraying of the throat with pneumococcus vaccine will not produce complete immunity against pneumonia in monkeys. Failure under these circumstances may be due to the fact that the monkey, by closing off the nasopharynx, prevents the vaporized vaccine from entering the trachea.
3. The immunity established by intratracheal injection of pneumococcus vaccine is probably cellular in character. Little or no protective substance against pneumococcus can be demonstrated in the serum of monkeys vaccinated by this method.

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SHIP FUMIGATION.

PRELIMINARY REPORT OF THE BOARD APPOINTED BY THE SURGEON GENERAL TO INVESTIGATE THE SUBJECT OF FUMIGATION OF SHIPS.

Because of the increasing requirements imposed by the quarantine laws of the United States and other countries, especially with reference to rodents, the Public Health Service has felt the need for further study of the whole subject of ship fumigation. For this reason the Surgeon General convened a board on March 1, 1922, consisting of a medical officer, a chemist, and an engineer to inquire into the subject and make recommendations.

In considering this matter the board arranged the work in two main divisions:

First. The investigation of the various gases which seem to offer advantages in ship fumigation, and experimentation regarding the production of these gases.

Second. The investigation of natural ventilation and artificial means of ventilation of ships after fumigation.

INVESTIGATION OF VARIOUS GASES FOR SHIP FUMIGATION.

The two gases which have been largely used for ship fumigation in the United States are hydrocyanic acid gas and sulphur dioxide. Each of these gases has certain advantages and disadvantages not